First of all I have to thank the organisers for the kind invitation. It is my pleasure to discuss with you one important let’s say topic which is related to the polyoma virus entity.
This slide shows you very briefly as a reminder that BKV belongs to the polyoma virus family. This virus is latently infected, the tubular cells, epithelial cells, endothelial cells, as well as macrophages and the clinical manifestation is not only linked to the nephropathy but is also linked to urethral structures, haemorrhagic cystitis as well as to the Guillan-Barré-Syndrome.

Slide 3

If we analyse during the last decade the main reason for organ failure within the first year of the kidney transplantation, we have to consider that BKV associated nephropathy is one of the first important factors which are linked to organ failure in the first year.
We have to state that around 3-10% of our organ failure within the first year is associated to an uncontrolled spreading of survivals of the BKV virus.

Slide 5

That is a little bit difficult to understand because if we analyse the infection process by this virus, we know that the majority of us are already infected. If we investigate normal healthy, then we find out that around 30-50% of them show transiently viruria as well as others transiently 10-20% viremia. But only let’s say 10% of them developed BKV nephropathy.

Slide 6
That leads to two important questions: one question is why do 10% of our kidney patients develop BKV associated nephropathy despite a high frequency of latent infection in kidney transplant patients as well as in healthys? The second important question is why have we observed an increasing incidence of this infection after transplantation during the last decade?

One important hypothesis or observation coming on the horizon is that obviously this infection is linked to any kind of change in the immune suppression regimen in the last decade as a risk factor.

Slide 7

Risk factors of BKVAN

<table>
<thead>
<tr>
<th>OR</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>FK 506</td>
<td>2.3</td>
</tr>
<tr>
<td>MMF</td>
<td>1.8</td>
</tr>
<tr>
<td>HLA class I mismatch</td>
<td>1.4</td>
</tr>
<tr>
<td>BK-viruria (&gt;10^4)</td>
<td>61.8</td>
</tr>
</tbody>
</table>

If we analyse the risks constellation for patients who have or who had a BKV associated nephropathy it is clearly shown by different groups that obviously FK in combination or not with MMF and in conjunction with and HLA class I mismatch is linked to a higher risk for BKV
nephropathy. But the suggestion behind this is that it is obviously related to a lack of adequate immune control as an important risk factor.

Slide 8

**Immunosuppressive regimen influence the risk of BKV nephropathy**

Retrospective analysis of treatment of BKV within 24 months of Organ Procurement Transplant Network data from 34,937 primary kidney transplant recipients 2003-2006

This paper published two year ago in Transplantation highlights this information from another perspective. This paper highlights it if you have a cyclosporine-based immunosuppressive regimen, if you treat the patients conservatively with azathioprine and not let’s say with more potent antimetabolites or if the patients are on mTOR inhibitor regimens, they have a lower risk for development of BKV associated nephropathy.

Slide 9

**Immunosuppression alone cannot explain BKVAN risk**

<table>
<thead>
<tr>
<th>Incidence of BKVAN</th>
</tr>
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<tbody>
<tr>
<td>kidney transplantation</td>
</tr>
<tr>
<td>kidney/pancreas Tx</td>
</tr>
<tr>
<td>liver transplantation</td>
</tr>
<tr>
<td>heart transplantation</td>
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</tbody>
</table>

Rabe et al. submitted
But the immunosuppression alone we think cannot explain the risk constellation for a special population among our transplant population because if we investigated liver transplant patients or even heart transplantation patients who suffer from let’s say kidney diseases that means they have an upcoming rise of creatinine, we never found any kind of BKV associated nephropathy pictures in the kidney.

Slide 10

**Immunosuppression alone cannot explain BKVAN risk**

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</table>

Kidney transplantation delivers a co-factor of BKVAN - intragraft inflammation

Randhawa et al., Larsen et al., Verne-Sempere et al., ATG 2005

That means obviously in kidney transplant patients, the kidney itself, the transplanted organ delivers a cofactor for this kind of let’s say disease, this is an intragraft inflammation.

Slide 11

**Risk factors of BKVAN**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>OR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FK 506</td>
<td>2.3</td>
<td>0.01</td>
</tr>
<tr>
<td>MMF</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>HLA class I mismatch</td>
<td>1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>BK viruria (&gt;10^5)</td>
<td>1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Donor age &gt; 60 y</td>
<td>3.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Acute rejection</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>CMV (reactivation)</td>
<td>2.1</td>
<td></td>
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</tbody>
</table>

intragraft inflammation supports BKVAN

If we look at the epidemiological data for risk, it is very impressive that the highest risk constellation together with the immunosuppression and the mismatch is related to the donor age, related to more frequent or severe acute infection and cytomegalovirus infection which means obviously that intragraft inflammation supports the reactivation of the virus itself and if severe virus spreading is badly controlled by the T cell response, then this it is linked to the damage of the organ.

Slide 12

**What are the specific clinical symptoms of BKVAN?**

- no any
  - (accidental finding in protocol biopsies)
- serum creatinine rise like in acute rejection
- histology similar to acute rejection (infiltrates etc.), but some specific events
- no response to antirejection therapy

![Acute rejection and BKVAN similar picture but completely different therapeutic strategies](chart)

![Need for specialised diagnostics](chart)

Now what are the specific clinical symptoms? It is very easy we don't have any clinical specific symptoms except a rise of serum creatinine, except that if we treat the patients because the histological findings are more or less similar to acute rejection we have no response to our rejection therapy. That means we need a specialized diagnosis.

Slide 13
This slide shows you very shortly that the histology is of course, the golden standard for the diagnosis but you see also that you have here mononuclear infiltrates which are more or less similar to acute infection but if you look here, you find such kinds of viral inclusions bodies and if you stain them with a special immune histology and stain for one important antigen of this virus, then you find the right diagnosis. This is the BKV associated nephropathy.

Slide 14

But again you have to be aware that we have a simple error.

Slide 15
That was very nicely described in the late 70s by Mackenzie. This might mimic an acute rejection phase episode I think it is a very difficult clinical decision and I’m very glad I’m a pathologist. He highlighted important information about this virus.

Slide 16

**Strategies for BKV Monitoring**

- urine cytology (Decoy cells)
- PCR (DNA/mRNA) urine/serum/plasma
  
  quantitative BKV-PCR (serum/urine) monthly for the first 6 mo. after Tx
  in case of graft deterioration
  quantitative BKV-PCR (serum) in case of therapy

  Cut-off:
  - > $10^7$ virus copies/ml urine or
  - > $10^4$ virus copies/ml serum

  virus specific immune response

We are now in a new century and we are very glad to have other possibilities to figure out patients on risk for BKV nephropathy. This is first urine cytology looking for decoy cells but the most important one is to analyse by a quantitative PCR the viral load either in urine or in plasma for the first let’s say 6 months after transplantation because this is the most vulnerable phase for reactivation of the virus in any case of graft deterioration or in case of any changes in the therapeutic regimen for let’s say stabilization of the infected graft.

Slide 17
Now, that leads us to the therapeutic options and we have to say that we don’t have any specific treatments at the moment. We have furthermore no randomised trials and moreover the treatment modalities are let’s say unknown, we don’t know when is the best time point to start with any kind of intervention and for how long we have to treat. The only thing we really know is that the success of our let’s say therapeutic intervention decreases with time meaning a small severe... the graft inflammation is as poor the graft outcome independent of our therapeutic strategy.

**Therapeutic Options**

- no any „specific” treatment available
- no any randomised trials published - „case reports”
- unknown treatment modalities (when to start ? how long to treat ?)
- success of „therapeutic” intervention decrease with time

As more severe the graft inflammation
As purer the graft outcome

Now, that leads us to the therapeutic options and we have to say that we don’t have any specific treatments at the moment. We have furthermore no randomised trials and moreover the treatment modalities are let’s say unknown, we don’t know when is the best time point to start with any kind of intervention and for how long we have to treat. The only thing we really know is that the success of our let’s say therapeutic intervention decreases with time meaning a small severe... the graft inflammation is as poor the graft outcome independent of our therapeutic strategy.

**Correlation between Severity of BKVAN and Clinical Outcome**

Analysis of 90 KTx patients with BKVAN (University of Maryland School of Medicine 1997–2003)

- A: Negligible inflammation or tubular atrophy interstitial fibrosis
- B1: Pathological changes in <25% of biopsy core
- B2: Pathological changes in 25-50% of biopsy core
- B3: Pathological changes in >50% majority of biopsy core
- C: End-stage BK-FyVAN

- correlation between histology grading (score) of BKVAN and graft survival (p=0.0002)
- low grade score is correlated with reduction / disappearance of BKV load as an indicator for “treatment” response (p=0.001)

This information or the statement is highlighted by two important papers from Cynthia Drachenberg and Hans Hirsch. They implemented a severity score of BKV associated nephropathy and what they highlighted is that if the patients have a high, severe score regarding the pathology that is closely linked to poor graft survival or in other words vice versa
if we have a low grade inflammation and we observe a disappearance of the BKV load, we can conclude that obviously this infection is under control by the T cells.

Slide 19

**Therapeutic Options**

- reduction of immunosuppression
- changing of immunosuppression ("switch")
- antiviral therapy (Cidofovir) (Vats et al., 2003; Kyperts et al., 2005)
- ivIg (Sener et al., 2006)
- leflunomid (William et al., 2005; Josephson et al., 2006)
- adoptive T cell therapy?

Efficacy not good validated (40% graft loss) (Walde et al., 2006)

Now, these are the therapeutic options. We can reduce immunosuppression, we can change the immunosuppression, we can use any antiviral therapies i.e. ivIg, leflunomid but we have also to say that the efficacy of all these let's say strategies are not well validated.

Slide 20

**Active BKV infection - what is the best therapy?**

Analysis of 52,989 renal transplant patients (UNOS data bank)

50,951 renal transplant patients with no BKV replication (controls)

&

2,061 renal transplant patients treated due to BKV reactivation

reduction/modification of IS or adjunctive therapy (Cidofovir, Leflunomid, ivIg)

Therapy outcome / Risk for the graft failure?

Shah et al., ATC 2009

This paper published two years ago investigated more than 2000 patients from the UNOS renal database regarding the efficacy of graft prevention in case of reducing the immunosuppression or using adjunctive therapy.
and he highlighted that the adjunctive therapy is obviously in this case in which the BKV associated nephropathy is established already not beneficial.

So therefore, the KDIGO guidelines implement 3 possibilities: one is a switching, one is a decreasing and one is a discontinuation.
We started let's say 8 years before with a strategy in case of BKV associated nephropathy to switch from TAC to CSA, from MMF to azathioprine
and the intention behind or the knowledge behind was that viral epitope recognition in the context of cross presentation is less susceptible to cyclosporine and moreover the functional activity of BKV specific T cells is not affected by cyclosporine.

Slide 26

Reconstitution of BKV-specific T-cells determines the decline of BKV load

This is one important information I want to share with you if you have a patient who had an upcoming viral load over time and you switch or reduce immunosuppression it is closely linked to the fact that you have a reconstitution of the BKV specific T cells and as a result of this you have a fast decline of the viral load and this is related to a time frame of around about 4 weeks after initiation this kind of change in the immunosuppression.

Slide 27
If we ask the question which population is related to this kind of control of the virus spreading then obviously the CD4 T cells which express an activation marker CD40 ligand are able to control the spreading of the virus.

Slide 28

<table>
<thead>
<tr>
<th>Adjunct therapy</th>
<th>Rationale for use</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| Leflunomide     | • in vitro antiviral effects  
• retrospective analysis (76 pats.) (Crisel et al., ATC 2011): no impact on reduction of BKV load  
• retrospective analysis Leflunomid vs. reduction of T8 (25 pats.); no impact on reduction of BKV load | • limited clinical data (conflicting)  
• cave: livertoxicity |
| Cidofovir       | • "global" antiviral activity  
• low dose application → reduction of viral load | • limited clinical data (conflicting)  
• cave: nephrotoxicity |
| ivIg            | • small case series (in combination with reduced T8 !!) normalisation of histology and stabilisation of graft function | |
| Quinolone       | • Quinolone show antiviral activities | • limited clinical data |

Now a few words on leflunomid, cidofovir and ivIg. Leflunomid shows *in vitro* antiviral effects but no data exists that in human beings we have a reduction of the viral load. Cidofovir if we use it in a low dose is able to reduce the viral load without prominent nephrotoxicity ivIg is able to normalise histology and stabilize the graft function.

Slide 29
In conclusion BKV associated nephropathy is a relevant differential diagnosis of graft injury, the viral load seems to be as a prognostic value. A BKV load is essential for pre-emptive therapy of BKV nephropathy.

Slide 30

Conclusions

1. BKVAN is a relevant differential diagnosis of graft injury
2. BKV load seems to be of prognostic value
3. BKV load is essential for pre-emptive therapy of BKVAN
4. Immunosuppression and renal inflammation are synergistic key factors of BKVAN risk
5. Immunopathogenesis dominates BKVAN, and weaning immunosuppression might support immune reconstitution syndrome
6. Quality and quantity of T-cell response is associated with allograft outcome in BKVAN

The immunosuppression and renal inflammation are synergistic effectors. The immunopathogenesis dominates the BKV associated nephropathy and weaning of the immunosuppression might support immune reconstitution syndrome the quality as well as the quantity of T cell response is associated with allograft outcome.

Slide 31
Conclusions

1. BKVAN is a relevant differential diagnosis of graft injury
2. BKV load seems to be of prognostic value
3. BKV load is essential for pre-emptive therapy of BKVAN
4. Immunosuppression and renal inflammation are synergistic key factors of BKVAN risk
5. Immunopathogenesis dominates BKVAN, and weaning immunosuppression might support immune reconstitution syndrome
6. Quality and quantity of T-cell response is associated with allograft outcome in BKVAN
7. Modification of immunosuppression (MIS) is effective and safe approach
8. BKV load decline appears within 4 weeks after reconstitution of BKV-specific T-cell immunity
9. Reconstitution of T-cell immunity is clearly correlated with MIS

and the modification of immunosuppression is effective, is safe, is related to a decline of the viral load within 4 weeks after reconstitution of a BKV specific T cell immunity and the reconstitution that means the better control by T cell immunity is clearly correlated with this kind of modification of immunosuppression.

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Acknowledgement

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